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PATENT SPECIFICATION (11) 1 551 518

(21) Application No. 13485/77 (22) Filed 30 March 1977
(31) Convention Application No. 51/035471
(32) Filed 31 March 1976
(31) Convention Application No. 51/035468
(32) Filed 31 March 1976 in
(33) Japan (JP)
(44) Complete Specification published 30 Aug. 1979
(51) INT CL² C07G 7/02//C13K 1/06 11/00 (C07G 7/02 7/028)
(52) Index at acceptance
C3H 200 241 H1
C2C 20Y 306 30Y 321 32Y 366 367 373 37Y 620 757 RL
C2S

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(54) IMMOBILIZED ENZYME COMPOSITIONS AND PROCESS FOR PRODUCING THE SAME

(71) We, DENKI KAGAKU KOGYO KABUSHIKI KAISHA, a company organised under the laws of Japan, of 1-4-1 Yuraku-cho, Chiyoda-ku, Tokyo, Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to immobilized enzyme compositions useful for industrial applications.

Immobilization of enzymes is often very advantageous in industrial applications. One of the industrially important merits is that the immobilized enzymes can be packed in a column type reactor to serve for a continuous enzyme reaction over a long period of time.

Various techniques for the enzyme immobilization have thus far been proposed, particularly with regard to glucose isomerase which, in the immobilized state, has a great importance in industrial applications, see for example U.S. Patent Nos. 3,821,086, 3,915,797 and 3,980,521.

This invention seeks to provide a development over the prior art immobilization techniques, more particularly to provide a process for producing immobilized enzyme compositions of good physical strength, with high enzymatic activity and a reasonable stability and showing a sufficient liquid penetration when packed in an industrial scale reactor.

This invention stems from the prior invention of our U.K. Patent No. 1,441,543 wherein an immobilized enzyme of high enzymatic activity and excellent stability can be produced by immobilizing a biologically active system into a substantially water-insoluble anion-exchange resin containing a quaternary pyridine ring in the polymer unit thereof, that is, a reactive high polymer which is insoluble in water but hydrophilic and obtained by quaternization of a vinylpyridine copolymer. The present invention seeks to improve the physical strength of the immobilized enzyme which has been a drawback of the above invention.

In accordance with one aspect, the present invention provides a process for producing an immobilized enzyme composition, comprising the steps of:

(1) reacting a biologically active enzyme system with a substantially water-insoluble anion-exchange resin having a quaternary pyridine ring in the polymer unit thereof, thereby to coagulate and immobilize said enzyme and/or enzyme-containing microbial cells,

(2) molding the reaction product to step (1), in a wet state, in an extrusion molding machine into a shaped form, and

(3) drying the molded product.

Because of the molding step, the immobilized enzyme compositions of this invention are of improved physical strength, which make them particularly useful for employment as catalysts in continuous, industrial scale, enzyme reactions. For

example, a great pressure loss is often experienced with large-scale industrial reactors or reactors using industrial materials of high density and viscosity, such as for the hydrolysis and isomerization of dextrose, and therefore it is desirable that catalysts to be used therein should be strong enough to suppress such a pressure loss.

By using an elevated temperature for the drying, the chemical reaction between the anion-exchange resin and the enzyme substance can be accelerated, thus reducing the risk of undesirable degradation of the enzyme substance.

In the preferred embodiments, the immobilized enzyme composition is molded so as to form pellets and if desired, there may be a further pelletizing in a pelletizing machine following extrusion. It is particularly preferred to form pellets of a size in the range of 100 micron to 2 mm. An advantage of forming pellets is that the efficiency of the drying step is increased, whereby the amount of heat import needed in the drying step is reduced and with it, the degradation of the enzyme substance.

In a particular embodiment of the invention, further advantages can be attained by the addition of a bifunctional crosslinking agent capable of reacting with the anion-exchange resin or the enzyme substance.

While the above crosslinking agent may be effectively added at any stage of the process, the addition can be rendered more effective by supplying heat energy in the course of the drying, whereby the physical strength and the stability of the catalysts, that is, immobilized enzyme compositions, is enhanced.

Some of the advantageous features obtainable according to this invention are summarized as follows:

Since the compositions can substantially consist of the enzyme substance, high enzymatic activity can be provided and, in addition, pelletized catalysts of any desired shape and size can be produced by mechanical molding by suitably controlling the molding conditions.

In use, this enables a reaction to be maintained at a desirably high rate, by supplying a reactant material at a high feed rate into an industrial scale reactor packed with the immobilized enzyme composition according to the invention. Moreover, since the catalyst may be chemically crosslinked and it is mechanically molded, it can possess a high physical strength and provide an excellent penetration to the liquid passing through the reactor, thereby to reduce pressure losses and to ensure stabilized operation.

In additon, since mechanical molding and chemical crosslinking improve the chemical stability, there can be obtained various other advantages which were not present in the conventional processes, e.g. less deactivation of the enzyme even during continuous long use and less effects of impurities.

Further, it is possible to immobilize not only an individual enzyme and/or enzyme-containing microbial cells but also a plurality of enzyme and/or enzyme-containing cells in combination, which therefore provides the possibility of a wide variety of applications.

Detailed Description of the Invention

1) Biologically Active Enzyme Substance

Typical enzymes and/or enzyme-containing microbial cells which may be used in this invention include, for example, enzyme (A) or enzyme-containing microbial cells (C) individually; enzyme (A)+enzyme (B); enzyme (A)+enzyme-containing microbial cells (C)+enzyme-containing microbial cells (D).

Representatives of the enzymes usable in the process of this invention include; glucose isomerase, lactase, aminoacylase, penicillin amidase, glucose oxidase, urease, phenoloxidase, catalase, invertase, alcohol dehydrogenase, lysozyme, steroid dehydrogenase, steroid hydroxylase, lactate dehydrogenase, amino acid oxidase, tyrosinase, ribonuclease, lipase, cellulase, α-amylase, glucoamylase, melibiase, asparaginase, β-amylase, maltase and cyclodextranase, and also such enzymes may be contained in microbial cells. As noted above a plurality of enzyme substances may be used in combination.

2) Carriers

The anion-exchange resin containing a quaternary pyridine ring in the molecule which is used as the carrier for the enzyme substance in this invention is a water insoluble but highly hydrophilic reactive high polymer produced by reacting a reagent capable of quaternizing the nitrogen atom in the pyridine ring in a copolymer which is produced by the copolymerization of vinylpyridine or its derivatives with one or more of monomers capable of copolymerizing therewith and selected from aromatic vinyl compounds, ethylenically unsaturated compounds and unsaturated diene series compounds.

Examples of anion-exchange resins suitably used in this invention include those having a quaternary pyridine ring in the molecule and obtained by reacting a quaternizing reagent such as alkyl halide or alkyl dihalide with a vinylpyridine-styrene-divinylbenzene copolymer, a vinylpyridine-methylmethyacrylate - divinylbenzene copolymer, a vinylpyridine -

polyethyleneglycol - dimethacrylate copolymer, a vinylpyridine - styrene block copolymer, or a vinylpyridine - methylmethacrylate block copolymer.

3) Crosslinking Agents

The preferred polyfunctional crosslinking agents usable in this invention are those compounds capable of reacting with functional reactive groups such as quaternary pyridine groups, and pyridine groups in the carrier, and hydroxyl, carboxyl, phenol, amino, mercapto groups or the like in the enzymes and/or microbial cells and they, desirably, include aldehydes, acid anhydrides, epoxides, halides, isocyanates and the like. More specifically, preferred crosslinking agents include dialdehyde starch, glutaraldehyde, ureaformaldehyde condensate, succinic anhydride, maleic anhydride copolymer, epichlorohydrin, epoxy resin, s-chlorotriazine, amino dichlorotriazine, toluene diisocyanate, dichlorobutene, and Woodward's reagent. Dialdehyde starch is particularly effective.

Since most of the crosslinking agents generally react with the reactive groups in the enzymes to reduce the enzymatic activity (although they can improve the physical strength of the enzymes), this fact should be borne in mind in considering the employment of a highly reactive crosslinking agent. However, the use of dialdehyde starch in combination with carriers containing a quaternary pyridine ring can improve the catalyst strength with no substantial reduction in the enzymatic activity.

Production of Immobilized Enzyme Catalysts

1) Coagulation reaction between enzymes and/or enzyme-containing microbial cells and the carrier

The reaction between the enzymes and/or enzyme-containing microbial cells and the carriers is, desirably, effected in a liquid phase, an aqueous dispersion of the carriers being added to and mixed with the enzyme and/or enzyme-containing microbial cells which has been dissolved or dispersed in water at their respective optimum pH ranges, to have them immobilized through coagulation.

A coagulated phase reaction is further desired wherein the aqueous dispersion of the carriers is added to and mixed with the enzyme and/or enzyme-containing microbial cells in a dried state to immobilize them through coagulation.

Preferred carriers used herein are finely divided powders of less than 0.01 mm particle diameter which are desirably produced by powdering in the course of the production step for the carriers or powdering the resulted carriers in a grinding machine or a ball mill. Since the carriers are highly acidic, it is desired to use an aqueous dispersion of the carriers adjusted to a pH range between 5.0—9.0.

An appropriate addition ratio between the enzymes and/or microbial cells and the carriers is 1:0.005—1:0.5 and, more preferably, 1:0.05—1:0.2 in each of their dried weights. Outside the above range, the immobilizing reaction through coagulation may be insufficient or excess carriers may cause undesired effects on the enzymatic activity.

The immobilizing reaction through coagulation above described is, desirably, effected at a temperature of from 0—30°C, considering the degradation of the enzyme substance.

The immobilized enzymes and/or immobilized microbial cells are molded in an extrusion molding machine whilst in a wet condition. It is preferred to adjust the water content of the immobilized product to from 25—80% by weight in order to facilitate the extrusion. A suitable water content within this range can readily be attained by controlling the operating conditions of a centrifugal separator or a filter press in the case when the immobilizing reaction is accomplished through coagulation in the liquid phase, or by the addition of a suitable amount of water prior to the molding when the immobilizing reaction is effected in a coagulated phase.

2) Molding of Wet Immobilized Products

Molding of the wet immobilized products is effected in an extrusion molding machine corresponding to a desired final shape of the catalyst.

The catalyst desirably has a final ellipsoid or rounded head shape and a thickness or diameter of from 1μ—10 mm, preferably, 100μ—2 mm, considering a well balanced combination between initial enzymatic activity and penetration to liquid. In a preferred molding method employed in this invention, the wet immobilized products are molded in an extruder having a 0.1 mm—2 mm screen. In the extrusion, the extruder used requires no special structure and a conventional type of extruder can suffice for the above purpose.

For further pelletizing the molded products, if desired, a rotary pelleting machine is advantageously used. By the optimum selection of the revolutional number and time, the catalyst can be formed into a shape and a size best suited for its intended use. It is also desired that the molding operation is effected in as short a time and at as low a temperature (0—

25°C) as possible to prevent the degradation of the catalyst.

It is effective in the extrusion molding, for the improvement in the extrusion property, stabilization of the shape and size of the molded pellets, as well as for giving porosity to the catalyst, to add various fillers such as an inorganic substance, for example, diatomaceous earth, silica; and aqueous dispersions of e.g. starch, dextrin, sodium alginate, carboxymethylcellulose, polyvinylalcohol, cellulose acetate, ethylene-vinylacetate copolymer, or sodium polyacrylate.

3) Drying of the Molded Products

The molded products produced as described above should be dried rapidly. It is particularly preferred to effect drying by blowing air at 40—80°C for 15 minutes—20 hours, although alternatively the drying can be effected, for example, with a shelf type drier in a stationary state or with a fluidized bed drier in a fluid state. Drying at higher temperature for a long time will result in a remarkable reduction in the enzymatic activity and lower temperature drying takes a long time, which can result in degradation of the enzyme and/or microbial cells.

4) Crosslinking of the Catalyst

The optional crosslinking reaction for the enzymes and/or enzyme-containing microbial cells can be effected at any stage in the production process of this invention. In a preferred embodiment, a crosslinking agent is added in solution of any desired concentration appropriate to the property of the agent at the time the coagulating immobilization is taken place, when the enzymes and/or enzyme-containing microbial cells are reacted with the carriers.

Where dialdehyde starch is used as the crosslinking agent, it is added, preferably, as a 1—20% by weight aqueous solution so as to provide 0.1—10% by weight of dialdehyde starch based on the solid contents of the enzyme and/or enzyme-containing microbial cells.

In a alternative preferred embodiment, solution of the crosslinking agent is added at the same time as the wet immobilized products, after the coagulating immobilization, are molded in the extrusion molding machine.

In another preferred embodiment, the wet products molded in the extrusion molding machine are contacted with the solution of the crosslinking agent.

In a further preferred embodiment, the solution of the crosslinking agent is contacted with the molded products after they have been dried.

Where dialdehyde starch is used as the crosslinking agent, it is preferred to contact the molded products in a dried state with an 0.5—20% by weight aqueous solution of the dialdehyde starch for from five minutes to five hours. In the above case, strength and stability of the catalysts can further be improved by drying them in the manner described after the completion of the crosslinking reaction.

Solvent for the crosslinking agent should be selected depending on the solubility of the agent, and where a water insoluble crosslinking agent is used it is dissolved into a mixed solution of a nonaqueous organic solvent and water to prepare a solution. Such a mixed solvent of the nonaqueous organic solvent and water is used also for a water soluble crosslinking agent, if it is highly reactive, to moderate the reactivity thereof because it may possibly attack the active groups in the enzymes to cause deactivation.

Preferred examples for the non-aqueous organic solvents include acetone, methanol, ethanol, propanol, isopropanol, tetrahydrofuran, dioxane, dimethylformamide, dimethylsulfoxide, glycerine, and polyethyleneglycol.

The amount and the concentration of the crosslinking agent used for the enzymes and/or enzyme-containing microbial cells have no particular limits since they are varied depending on the effects of the solubility and reactivity of the crosslinking agent, as well as the activity and strength desired for the catalyst.

The pH range for the solution of the crosslinking agent should be determined based on the enzymatic activity and the crosslinking effect and adjusted suitably to between 4.0—9.0, more preferably near to a neutral point.

It is desired to effect the crosslinking reaction at as low a temperature and in as short a time as possible in order to minimize the degradation of the enzyme substance.

Since the immobilized enzyme catalysts thus obtained preferably consist essentially of the enzymes and/or enzyme-containing microbial cells and a minor amount of carrier, high enzymatic activity per unit weight of the catalysts can be realized and, in addition since the catalysts have a dense and solidified structure as a result of the mechanical molding and optionally chemical crosslinking reaction, the penetration by liquids in use is good while, in referred embodiments, pressure losses in use caused by degradation of the catalyst are not significant.

The invention is illustrated by the Examples which follow and in which all percentages are by weight unless otherwise stated.

Example 1

1) Preparation of Immobilized Catalyst

A monomer mixture consisting of 48 mol% styrene, 50 mol% 2-vinylpyridine, 2 mol% divinylbenzene was added to water, which contained 5% sulfate salt of polyoxyethylene alkylphenolether (Nissantrax K, as commercial name) as an emulsifier in a monomer to water ratio of 1:1/2 by volume, emulsified therein and, thereafter, emulsion polymerized at 60°C using potassium persulfate as a catalyst.

10 hours after the reaction, an excess amount of acetone was added to the reaction solution to precipitate the resulting polymer in powder state, and the precipitate was washed with warm water several times and then dried. the polymerization degree was 95%.

The copolymer thus obtained was quaternized in a pressurized gas phase at 80°C using methylbromide as a quaternizing agent. 50 g of the quaternized styrene - 2 - vinylpyridine - divinylbenzene random copolymer (anion-exchange capacity of 3.2 meq/g) was dispersed in 250 ml water and the resulting suspension was adjusted to pH 7.0 by using 0.1 N NaOH.

Then, an aqueous dispersion of commercially available microorganic cells having glucose isomerase activity, produced from Streptomyces species (enzymatic activity of 1600 GIU/g; about 40% solid contents); was disrupted by a sonic oscillator and, thereafter, the supernatant liquid was condensed and then freeze-dried. 50 cc of the carrier dispersion prepared as above was added to 100 g of the glucose insomerase (15000 GIU/g of activity) and well compounded to prepare coagulated immobilized products.

50 ml of 5% aqueous solution of dialdehyde starch prepared by treating starch with periodic acid (containing 10% of sodium sulfite based on solid content: pH 6.0) was then added to the above coagulated immobilized products and well compounded at room temperature.

The compounded products were subjected to extrusion molding at ambient temperature using a single screw front surface extruder having a 1.0 mm ϕ screen. The extrudate strip of 2—10 cm width was cut to a 1 mm length and then dried in a fluidized bed drier at 60°C for one hour. The immobilized enzyme catalyst thus obtained possessed glucose isomerase activity of 4800 GIU/g.

2) Continuous Enzyme Reaction

100 g of the immobilized enzyme prepared as described in (1) above, was placed in a beaker containing 1 litre of distilled water and swelled for about one hour. It was then packed in a jacketed glass column of 2.5 cm diameter and 80 cm height kept at 60°C and, immediately thereafter, 59% by weight of an aqueous solution of glucose (pH: 8.0, and containing 5×10^{-3} M/l $MgSO_4.7H_2O$) was supplied continuously from above the column at a flow rate of 1500 ml/Hr. to initiate the reaction.

Pressure gauges were set at the upper and the lower portions of the column so as to measure the pressure difference. The reacted solution was taken out from the lower portion of the column and the isomerization rate was given by the determination of the resulting amounts of fructose by using a polariscope. The results are shown in Table 1.

TABLE 1

Days	Flow rate (l/Hr)	Yield of fructose (%—solid content)	Pressure loss (kg/cm^2)
2	1500	44.5	0.03
5	1500	47.3	0.03
10	1500	45.4	0.03
20	1500	46.1	0.03
30	1500	43.0	0.03
40	1500	42.2	0.03
50	1500	41.0	0.03

The enzymatic activity of glucose isomerase was measured on the basis of Takasaki's method (reported in Agricultural Biological Chemistry vol. 30, 1248 pp (1966)) wherein activity units were indicated by assuming the unity, 1 GIU/g, to represent production of 1 mg of fructose from 1 g of a given enzymatic composition under the reaction conditions of 70°C for 10 minutes.

Example 2

1) Preparation of Immobilized Catalyst

Through 1000 g of the same commercially available microorganic cells possessed of glucose isomerase activity (1600 GIU/g; about 40% solid content) as used in (1) in Example 1 above, was passed 250 ml of an aqueous solution containing 50 g of the same quaternized random copolymer of

styrene - 2 - vinylpyridine - divinylbenzene (pH 7.0) as prepared in Example 1 while compounding in a kneader. After compounding for about 15 minutes at room temperature (25°C), 25 ml of a 10% aqueous solution of dialdehyde starch (pH 6.0) used in Example 1 was added and compounding was effected for a further 15 minutes.

The wet immobilized microorganic cells were then subjected to extrusion molding at ambient temperature in a twin screw horizontal extruder having a 0.5 mm ϕ screen and the extrudates were pelletized in a rotary pelletizer under the operation conditions of 750 rpm and 30 seconds/batch. The resultant pellets were dried by a fluidized bed drier for one hour at 60°C. The catalyst had an activity of 1030 GIU/g.

2) Continuous Enzyme Reaction.

Using 100 g of the catalyst prepared in (1) above, glucose isomerization was conducted by the same method as in Example 1—(2). The results are shown in Table 2.

Also in this reaction, the resulting products were quite colorless and of a high quality. In addition, since the pressure loss is extremely low, the operation can be performed with safety.

TABLE 2

Days	Flow rate (ml/hr.)	Yield of fructose (% solid content)
2	480	41.5
5	500	42.5
10	470	42.7
20	470	40.5
30	470	38.3
40	470	37.0
50	470	36.0

Example 3

1) Preparation of Immobilized Catalyst

50 g of a quaternized random copolymer of styrene - 2 - vinylpyridine - divinylbenzene as prepared in Example 1—(1) was dispersed in 5 l of water and the pH of the resulting suspension was adjusted to 7.0 with 0.1 N NaOH.

The, 1000 g of the same microorganic cells possessed of glucose isomerase activity as used in Example 1 (1) was suspended in 10 l of water, to which the whole of the copolymer dispersion described above was added while stirring at room temperature (20—25°C). After a 30 minute agitation, they were dewatered in a centrifugal separator to obtain immobilized wet microorganic cells. The yield was 1250 g (68% water content).

200 ml of a 2% aqueous solution of carboxymethylcellulose (commercially available as food additives) was added thereto and thoroughly compounded to a water content of about 72.5%.

The immobilized wet microorganic cells thus produced were then subjected to extrusion molding in a twin screw horizontal extruder having a 0.8 mm ϕ screen. The extrudates were pelletized in a pelletizer under the operation conditions of 500 rpm and 60 seconds/batch and the resulted wet pelletized products were dried for 20 hours using a blowing drier maintained at 50°C.

Then, 100 g dried pellets were added to 100 ml of a 2% aqueous solution (pH 6.0) at 5°C of the same dialdehyde starch as used in Example 1—(1) to react for about 30 minutes. After the reaction, the solid contents were filtered out, washed with pure water and then dried for one hour at 60°C using a fluidized bed drier. The catalyst obtained had 980 GIU/g of activity.

2) Continuous Enzyme Reaction

Using 100 g of the catalyst prepared as in (1) above, glucose isomerization was conducted by the same method as in Example 1—(2). The reaction was initiated at a reactant flow rate of 450 ml/hr. in which the yield of fructose (% solid content) was 43.5% at an initial stage and 40.3% after 30 days. Neither pressure loss nor the instability of the catalyst was observed at all in the course of the reaction.

Example 4

1) Preparation of Immobilized Catalyst

A quaternized random copolymer of MMA - 2 - vinylpyridine - divinylbenzene (anion-exchange capacity of 3.0 meq/g) was produced in the same way as in the production for the carriers described in the Example 1—(1) except for the use of methylmethacrylate (MMA) in place of styrene.

Then, while kneading 1000 g of the same commercially available microorganic cells possessed of glucose isomerase activity as used in Example 1—(1) in a kneader, 250 ml of an aqueous dispersion (pH 7.0 containing 50 g of the above carrier was added thereto and the kneading was effected for about a further 15 minutes at room temperature (25°C).

Then, the immobilized wet microorganic cells thus obtained were molded and dried in the same way as in Example 3—(1) to obtain dried pelletized products. 100 g of the pellets were then immersed into 500 ml of a mixed solution of water and acetone (pH 7.0, water/acetone ratio=1:4 by volume) containing 5% (w/v) glutaraldehyde and reacted for 30 minutes at 5°C. After filtration, the filtered products were washed with acetone several times and dried by blowing for two hours at 60°C. The catalyst had 730 GIU/g activity.

2) Continuous Enzyme Reaction

Using 100 g of the catalyst prepared as in (1) above, glucose isomerization was effected in the same manner as in Example 1—(2).

The reaction was initiated with a reactant flow rate of 380 ml/hr., in which the yields for fructose (% solid content) was 45.3% at an initial stage 42.5% after 30 days and 41% after 60 days. Neither pressure loss nor instability in the catalyst was observed at all throughout the reaction.

Example 5

1) Preparation of Immobilized Catalyst

50 g of the quaternized random copolymer of MMA - 2 - vinylpyridine - divinylbenzene as used in Example 4—(1) was dispersed in 250 ml of water in suspension and the pH was adjusted to 7.0.

Then, 100 ml of the dispersion of the above carrier was added to and well compounded with 100 g of commercially available glucoamylase (activity unit, 30000 U/g) to prepare coagulated immobilized products.

The immobilized products were then subjected to extrusion molding in a single screw front extruder haivng a 1.0 mm ϕ screen at an ambient temperature. The resultant extrudate strip was cut to about 1 mm length and, thereafter, dried at 60°C for one hour in fluidized bed drier. Then, 100 g of the pelletized products were treated with glutaraldehyde and dried under the same conditions as in Example 4—(1). The catalyst had an activity of 2500 U/g, measured as described later in this Example.

2) Continuous Enzyme Reaction

100 g of the immobilized enzyme catalyst prepared as in (1) above was swelled in water for about one hour and then packed into a jacketed glass column of 2.5 cm diameter and 80 cm height kept at 40°C. Immediately thereafter, a 35% solution of liquified potato starch (pH 4.8 Dextrose Equivalent of 13) was continuously supplied from the top of the column at a flow rate of 1000 ml/hr. to initiate the reaction. The reaction solution was sampled at a certain interval of time from the bottom of the column and the yields of glucose were determined according to the glucostat method. The results are shown in Table 3.

TABLE 3

Days	Flow rate (ml/hr.)	Yield of glucose (% solid content)
2	1000	97.0
5	1000	95.2
10	1000	94.6
15	1000	94.0
20	1000	93.6
30	1000	92.8
40	800	95.0
50	800	94.3

Glucoamylase activity: Yield of the reducing sugar was determined using a 1.2% solution of soluble starch (pH 4.5) as a basic reactant and according to the Somogyi-Nelson method (experimental methods in biochemistry, "Method for determination of reducing sugar" p10 published by Tokyo University Publishing Association). Activity units were indicated by assuming the unity, 1 unit/g, to represent liberation of reducing power corresponding to 100 μg of glucose in one minute from 1 g of the enzyme pellets.

Example 6

1) Preparation of Immobilized Catalyst

100 g of commercially available glucoamylase (activity unit of 30000 U/g) was dissolved in 100 ml of water. Then, 200 ml of a 10% acetone solution of maleic anhydride-vinylacetate copolymer (in 1:1 molar ratio) was gradually added to the above solution under agitation and the pH was adjusted to 7.0—8.0 with 1/10n NaOH. The reaction was conducted at 5—10°C. After the entire amount of the copolymer had been added, agitation was continued for a further 10 minutes and then freeze-drying was effected to obtain succinic glucoamylase.

Then, 50 ml of the same carrier dispersion as used in Example 5—(1) was added to and well compounded with the above enzyme and further mixed thoroughly with 50 ml of an aqueous solution of the same dialdehyde starch as used in Example 1—(1) at room temperature (25°C). The wet immobilized products were then molded and dried by the same methods as used in Example 4—(1) to prepare the catalyst. The catalyst had an activity of 2800 U/g.

2) Continuous Enzyme Reaction.

Using 100 g of the immobilized enzyme catalyst prepared in (1) above, a continuous saccharification of starch was effected in the same manner and under the same conditions as employed in Example 5—(2). The reaction was conducted for 50 days while setting the flow rate of the basic reactant to 1000 ml/hr. and, as a result, the yield of the resulting glucose was more than 94.0%.

Example 7

1) Preparation of immobilized catalyst

Aminoamylase-incorporating micro-organic cells produced by a deep culture of Aspergillus oryzae were subjected to spray-drying 100 g of the dried cells were used for the immobilization and catalyst formation in the same way as in Example

1—(1) to produce immobilized enzyme catalyst.

2) Continuous Enzyme Reaction

100 g of the above catalyst was swelled in water for about one hour and then packed into a jacketed glass column of 2.5 cm diameter and 80 cm height kept at 50°C. N - acetyl - DL - methionine solution (0.2 mol, pH 7.0 containing 5.0×10^{-4} mol $CoCl_2$) was passed down from the top of the column at a flow rate of 1000 ml/hr. The reaction solution flowing out from the bottom of the column was sampled at certain intervals to determine the amount of L-methionine by way of ninhydrine colorimetry. The results are shown in Table 4.

TABLE 4

Days	Flow rate (ml/hr.)	Yield of L-methionine (% to N-acetyl-L-methionine)
2	1000	100.0
5	1000	100.0
10	1000	100.0
15	1000	99.6
20	1000	99.4
30	1000	99.0
40	970	100.0
50	970	99.5
60	950	100.0

Example 8

1) Preparation of Immobilized Catalyst

100 g of commercially available aminoamylase (activity unit 25000 U/g) was used to produce immobilized aminoamylase catalyst by the same methods and under the same conditions as in Example 5—(1).

2) Continuous Enzyme Reaction

Using 100 g of the above catalyst, continuous production of L-methionine was effected by the same methods and under the same conditions as in Example 7—(2). The reaction was conducted continuously for 60 days while setting the flow rate of the basic reactant to 4 l/hr, and, as a result, the yield for the L-methionine (to N - acetyl - D - methionine) was more than 99.5%.

WHAT WE CLAIM IS:—

1. A process for producing an immobilized enzyme composition, comprising the steps of:

(1) reacting a biologically active enzyme system with a substantially water-insoluble anion-exchange resin having a quaternary pyridine ring in the polymer unit thereof, thereby to coagulate and immobilize said enzyme and/or enzyme-containing microbial cells,

(2) molding the reaction product of step (1), in a wet state, in an extrusion molding machine into a shaped form, and

(3) drying the molded product.

2. A process according to Claim 1, wherein the extrusion molding machine is a twin screw horizontal type extruder.

3. A process according to Claim 1 or 2, wherein the extrusion molding machine has a 0.1—2 mm screen.

4. A process according to any preceding claim, wherein the molding of said reaction products in step (2) is effected at a temperature of from 0—25°C.

5. A process according to any preceding claim, wherein the water content of the reaction product of step (1) is adjusted to from 25—80% by weight prior to charging to the extrusion molding machine.

6. A process according to any preceding Claim, wherein the molded products are dried in step (3), in air at 40°—80°C for from 15 minutes to 20 hours.

7. A process according to any preceding claim, wherein a cross-linking agent which is reactive through chemical bonding with said reaction product of step (1) is added thereto before, during or after the molding of said reaction product in step (2).

8. A process according to Claim 7, wherein the cross-linking agent is selected from glutaric aldehyde, condensation products of urea-formaldehyde, succinic anhydride, maleic anhydride copolymer, dialdehyde starch, epichlorohydrin, epoxy resin, cyanuric acid chloride, aminodichlorotriazine, toluene diisocyanate, dichlorobutene, and Woodward's reagent.

9. A process according to any preceding claim, wherein the reaction product of step (1) is molded in step (2) to form pellets, and wherein, optionally, the resulting pellets are subjected to further pellitization prior to drying in step (3).

10. A process according to Claim 9, wherein there is formed pellets having a size of from 100 microns to 2 mm.

11. An immobilized enzyme composition molded into shaped form comprising a biologically active enzyme substance bonded to a substantially water-insoluble anion-exchange resin having a quaternized pyridine ring in the polymer unit thereof.

12. An immobilized enzyme composition according to Claim 11 in the form of pellets.

13. An immobilized enzyme composition according to Claim 12, wherein the pellets have a size of from 100 microns to 2 mm.

14. A cross-linked immobilized enzyme composition according to any one of Claims 11—13.

15. An immobilized enzyme composition according to Claim 11 and substantially as described in any one of the Examples herein.

16. A process for producing an immobilized enzyme composition, according to Claim 1 and substantially as described in any one of the Examples herein.

17. An immobilized enzyme composition whenever produced by a process according to any one of Claims 1—10 or Claim 16.

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Printed for Her Majesty's Stationery Office, by the Courier Press, Leamington Spa, 1979
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.